

Applicant : Bonner-Weir et al.
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Page : 3

Attorney's Docket No.: 10276-029001 / JDP-044

REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

A substantive response to the action mailed October 22, 2001, along with a Petition for Extension of Time and the required fee, is being submitted under separate cover.

Attached is a marked-up version of the changes being made by the current amendment.

Applicants submit that the application as now in compliance with the sequence rules.

Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 3/28/02

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Version with markings to show changes made

In the specification:

Paragraph beginning at page 36, line 14, has been amended as follows:

It appears that Pref-1 protein expression in ducts may also mark undifferentiated pluripotent duct cells and be expressed transiently after Px. To test this hypothesis, semi quantitative RT-PCR analysis can be run for Pref-1 using the primers:

5'CCTTGTGCTGGCAGTCCTTTCC (SEQ ID NO:7)

3'TCTGTGAGGCTGACAATGTCTGC (SEQ ID NO:8)

for rat Pref-1 with a-tubulin for an internal control for samples from isolated common pancreatic ducts from rats 4, 12, 24 hrs and 2, 3, 7 days after Px and sham Px surgery as well as unoperated controls. Standardized conditions for linear amplification for this set of primers has already been prepared using techniques as in our previous studies. In parallel, immunostain for Pref-1/FA-1 can be performed using an anti-Pref-1 antibody. The advantage of the RT-PCR for the initial screening is that much information can be gleaned from a small amount of tissue or one experiment. The disadvantage is that heterogeneity is not accounted for and that only a few cells that are strongly positive for a gene may give a false interpretation. The second tier of analysis, immunostaining, overcomes these problems of interpretation of RT-PCR. Particular attention can be given to Pref-1 protein expression in the common pancreatic ducts and in the ductules in the focal areas of regeneration. If the expression pattern resembles that of PDX-1, then double immunostain for PDX-1 (nucleus) and Pref-1 (plasma membrane/cytoplasm) can be performed to confirm whether there is co-localization. It is unclear from the previous reports if Pref-1 is regulated at the transcriptional or posttranscriptional level. If it is, various mammalian members of the Notch family can be screened using RT-PCR.